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# BIOLOGICAL BULLETIN

## THE RELATION BETWEEN REGULATION AND FISSION IN PLANARIA.

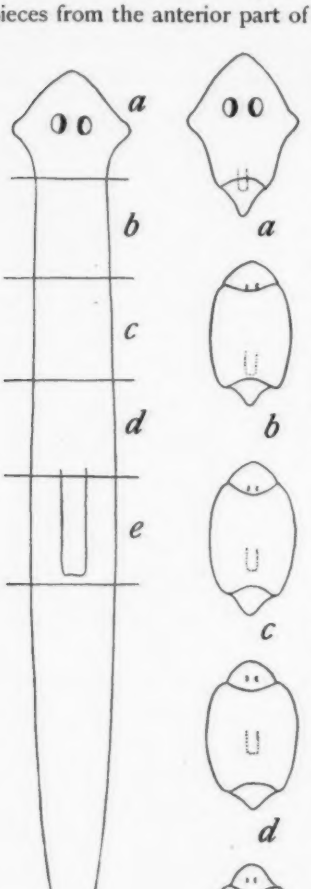
C. M. CHILD.

The data discussed below were obtained at various times during the last five years from two species of *Planaria*, *P. maculata* and an unnamed Californian species differing from *P. maculata* in color, length of "auricles" and length of pharynx (Fig. 8), but resembling it very closely as regards regulation and fission.

### I. THE POSITION OF THE PHARYNX IN PIECES FROM DIFFERENT LEVELS.

It has been known since Morgan's experiments on *Planaria maculata* that the position of the newly formed pharynx in a piece differs according to the level of the body from which the piece was taken. According to Morgan the new pharynx arises posterior to the middle of the piece in prepharyngeal pieces, its distance from the middle decreasing as the level of the piece approaches the pharyngeal region of the original animal; as regards postpharyngeal pieces, however, he states merely that the pharynx arises anterior to or near the middle of the piece. My own observations on prepharyngeal and pharyngeal pieces agree with his. Figs. 1 and 2, *a–e*, indicate this relation between level and pharyngeal position. The regulation of postpharyngeal pieces, however, presents a number of important features, which Morgan has not, so far as I am aware, described. If the whole postpharyngeal region be cut off as one piece, the new pharynx always arises a considerable distance anterior to the middle (Fig. 3) its position varying somewhat in different cases. If, however, this region be cut into several pieces (Fig. 4, *a*, *b*, *c*, *d*)

we find that the new pharynx appears anterior to the middle in pieces from the anterior part of the postpharyngeal region and at or very near the middle in pieces from the posterior portion (Fig. 5, *a*, *b*, *c*, *d*); in other words *the distance between the new pharynx and the anterior end increases with increasing distance from the old pharyngeal region.* By analogy with the prepharyngeal region we should expect just the opposite, but in only two cases out of more than a hundred have I seen it (Figs. 6 and 7, *a*, *b*, *c*). In these two cases *the distance between the new pharynx and the anterior end of the piece decreases with increasing distance from the old pharyngeal region.*




FIGS. 1, 2.

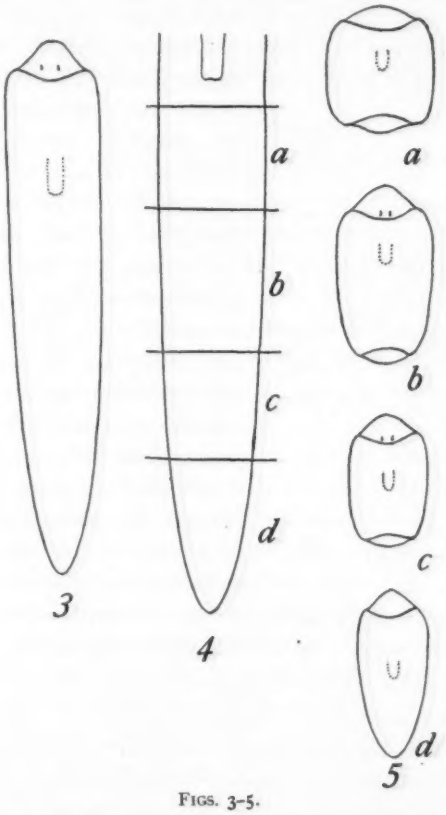
It is necessary here to refer very briefly to certain interpretations suggested in previous papers.[1] According to these suggestions the course of regeneration in a piece will depend very largely upon its past associations with the various functional

[1] "Studies on Regulation — IX., The Position and Proportions of Parts During Regulation in Cestoplana in the Presence of the Cephalic Ganglia," *Archiv f. Entwickelungsmech.*, Bd. XX., H. 1, 1905.

"Studies on Regulation — X., The Positions and Proportions of Parts During Regulation in Cestoplana in the Absence of the Cephalic Ganglia," *Archiv f. Entwickelungsmech.*, Bd. XX., H. 2, 1905.

"Contributions toward a Theory of Regulation — I., The Significance of the Different Methods of Regulation in Turbellaria," *Archiv f. Entwickelungsmech.*, Bd. XX., H. 3, 1906.

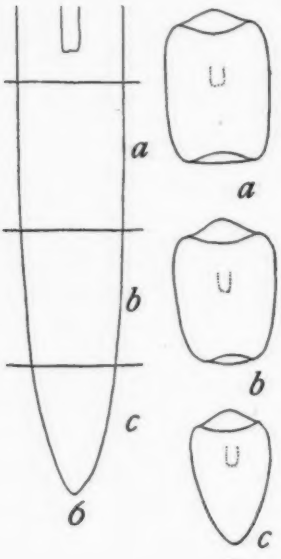
complexes of the whole, thus a piece from the anterior regions of the body is functionally much more "anterior" than a piece from the middle of the body; consequently in the new wholes formed from these pieces we shall find relative differences in size of various parts. In the "anterior" piece, for example, the anterior region will be much larger than the posterior and in the piece from the middle of the body the two will be about equal.




FIGS. 3–5.

In normal animals the pharynx is situated in the middle of the body but in those new animals formed from the pieces the physiological or functional middle does not coincide with the

structural middle: in anterior pieces the functional middle is near the posterior end; in pieces from the middle of the body it is near the middle, etc. The new pharynx appears at this functional middle or as we may more properly call it the physiological pharyngeal region. With this interpretation in mind the difference in position of the pharynx in the pieces *a–e*, Fig. 2, is readily understood.




FIGS. 6, 7.

Now we might expect that pieces from the postpharyngeal region would show relations similar in character but reversed, *i. e.*, a decrease in size of the new prepharyngeal region with increasing distance from the pharynx. As a matter of fact just the opposite occurs (Figs. 4, 5, *a*, *b*, *c*, *d*) except in very rare cases (Figs. 6 and 7) so that the prepharyngeal and postpharyngeal regions are usually equal in size in pieces from the extreme posterior end.

We must conclude from this relation that pieces such as *c* and *d* in Fig. 5 are physiologically more anterior than pieces *a* and *b*. I believe that the occurrence of fission affords a very simple explanation of these facts. It is highly improbable that fission occurs in these forms without some physiological preparation even though changes in structure may not be visible. Various authors have noted that fission in certain other turbellaria is initiated by changes in the nervous system leading to the development of new cephalic ganglia. If such changes are going on in this postpharyngeal region in *Planaria* there is no difficulty in understanding why the new prepharyngeal region should be longer in these pieces than in those farther anterior. Moreover,

I do not believe that any other explanation of these remarkable facts can be found.

The two exceptional cases noted above (Figs. 6, 7, *a*, *b*, *c*) were from worms captured through the ice in January when food was scarce and activity slight on account of the low temperature and hence in all probability no preparation for fission existed. These cases only serve, therefore, to render more probable the interpretation given above.

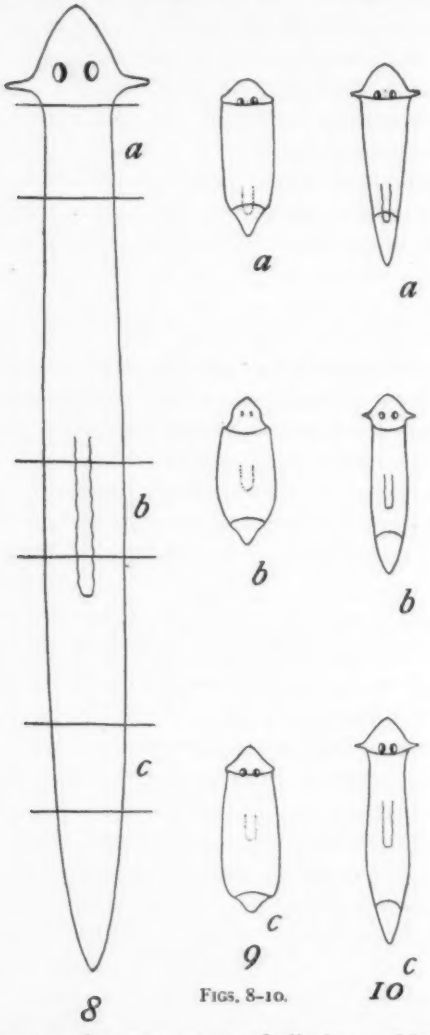
In *P. simplicissima*, which is not known to undergo fission the relative position of the pharynx in pieces as from different levels is, according to Morgan, similar to that in the winter specimens of *P. maculata*.

## II. The Size of the Head at Different Levels.

In 1901 I first noticed that the new head formed was larger in pieces from the anterior part of the prepharyngeal region and from the posterior half or two thirds of the postpharyngeal region than in pieces from the middle region of the body. Moreover, in the pieces from the middle region a considerable amount of new tissue is formed posterior to the eyes while in the anterior and posterior regions the eyes lie almost in the plane of the cut surface.

If we cut the whole body into equal pieces and compare them we find the size of the head decreasing and the distance between the eyes and the cut surface increasing as we proceed posteriorly toward the old pharyngeal region. Pieces just anterior or posterior to or in the pharyngeal region are much alike, all forming relatively small heads and relatively long "necks" of new tissue. In the postpharyngeal region, however, the size of the head increases and the length of the neck decreases until in the posterior half of the postpharyngeal region the head is about as large as in anterior pieces and the eyes appear in the plane of the cut. Both species of *Planaria* are similar in this respect. Figs. 8, 9 and 10, *a*, *b*, *c*, illustrate the case under consideration. Fig. 8 indicates the regions from which the pieces *a*, *b* and *c* were taken; in Fig. 9, *a*, *b*, *c*, the difference in size of the head and length of the "neck" in the new tissue is shown at an early

stage of regulation and in Fig. 10, *a*, *b*, *c*, at a later stage. These figures as well as all my conclusions on this point are based on




FIGS. 8–10.

careful and repeated measurements of all pieces, although the difference is sufficiently striking without such aids.

I believe that these differences are also indicative of the physiological conditions at different levels. In previous papers[1] I have suggested that the relation between redifferentiation and regeneration proper depends upon the physiological differentiation of parts. As the difference in physiological or functional specification between the part removed and the part remaining increases the amount of redifferentiation decreases and that of regeneration increases up to a certain limit and vice versa. To take a concrete case: the more "head-like" in the physiological sense the anterior portion of a piece of *Planaria* is, the smaller the amount of new tissue formed at this end and the greater the amount of redifferentiation. Now the anterior ends of pieces from the anterior regions of the body of *Planaria* and those from the posterior half of the postpharyngeal region are visibly more "head-like" than those of pieces from the middle region. This is evident from the degree and character of their activity. Correspondingly we find that only the part anterior to the eyes is formed by regeneration, the "neck" and in some cases the auricles themselves being formed by redifferentiation in the pieces from anterior and posterior regions (Figs. 9, *a*, *c*, and 10, *a*, *c*). On the other hand, in the pieces from the middle region not only the head but a longer or shorter "neck" is formed by regeneration.

The difference in size of the heads furnishes more evidence along the same line. The greater the relative intensity of a given functional complex the larger the relative size of the structural complex which represents it. Hence the more "head-like" a given piece is the larger the relative size of the head formed and vice versa (cf. Figs. 9, *a*, *b*, *c*, and 10, *a*, *b*, *c*).

The conclusions from this line of evidence are the same as those reached from a comparative study of pharyngeal position *viz.*, that in the posterior half or two thirds of the postpharyngeal region a marked increase in the functional conditions charac-

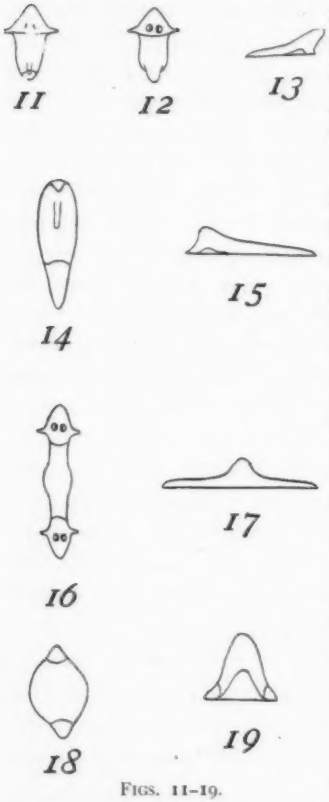
[1] "Studies on Regulation — VIII., Functional Regulation and Regeneration in Cestoplana," *Archiv f. Entwickelungsmech.*, Bd. XIX., H. 3, 1905.

"Contributions toward a Theory of Regulation — I., The Significance of the Different Methods of Regulation in Turbellaria," *Archiv f. Entwickelungsmech.*, Bd. XX., H. 3, 1906.

teristic of the anterior region occurs — that is the change is due to the development in this region of a new "head" physiologically speaking.

### III. Regulation in Very Small Pieces.

By the use of pieces approaching the minimal size in which regulation is possible, we have a means of determining the physiological potences of the various regions more exactly than by any other method. I have recorded the full history of a large number of species of this kind from both species of *Planaria*, though mostly from the Californian.




Figs. 11–19.

Worms of rather large size were selected and cut into eighteen to twenty pieces by transverse or nearly transverse cuts. Each piece was numbered and isolated and observed as an individual. The results of these experiments are rather remarkable. The pieces from the region immediately posterior to the old head produce a large head and a very small posterior end with the pharynx near the latter (Fig. 11) or sometimes no pharynx, *i. e.*, these pieces are so exclusively "anterior" physiologically speaking that sometimes they are incapable of giving rise to the pharyngeal and postpharyngeal regions at all, and in any case these regions are small. The pieces behind these show a smaller head and larger posterior end with the pharynx approaching the middle and give rise to normal animals. But a region including the old pharynx and extending for a short distance anterior and posterior

to it affords a remarkable variety of results in these small pieces. Five different methods of regulation occur: (1) The piece may form a normal animal; (2) it may be fully used up in the formation of a head and anterior region without a pharynx or postpharygneal region (Fig. 12). In such cases a dorsal thickening occurs in the posterior region (Fig. 13, optical section in median plane); (3) the piece may go entirely to the formation of a posterior region without head and with or without pharynx (Fig. 14). Such pieces bear a dorsal thickening in the anterior region (Fig. 15); (4) the piece may form a head at each end (Fig. 16). In these cases there is a dorsal thickening in the middle (Fig. 17); (5) the pieces may form a tail at each end (Fig. 18). Such pieces acquire after two or three weeks the outline shown in Fig. 19 in consequence of the opposed activity of the two posterior ends.

The cases 3 and 4 have been described by Morgan and others but so far as I am aware cases 2 and 5 have not been recognized as what they actually are, though they have undoubtedly been seen by many observers and grouped with abnormalities. The pieces from the postpharyngeal region almost without exception produce normal animals. Occasionally a case of double heteromorphic heads like Fig. 16 occurs very near the extreme posterior end of the body, often in next to the last piece.

Thus the middle region as compared with the anterior and posterior regions appears to be indifferent; pieces from it may form single or double anterior parts, or single or double posterior parts, or normal animals. The different possibilities do not occur in any definite order; a single or double "tail" is as likely to follow a double "head" as is a normal animal and *vice versa*. Neither does the presence of the old pharynx play any part in the production of these various results, for the region where they occur most frequently begins some distance anterior to the pharynx and ends some distance posterior to it.

I believe that we must consider this region as physiologically indifferent, *i. e.*, neither "anterior" nor "posterior" in the functional sense to any marked degree. In consequence of this indifference the actual course of regulation must depend on slight chance internal differences in the pieces. If one end becomes pbysiologically dominant the piece may give rise to a single

"head" or a single "tail." If both ends maintain their independence a normal animal or double "heads" or double "tails" may result according as the physiological conditions are different or alike. The dorsal thickening in these cases of partial animals represents that region of the body toward which the intestinal contents are forced during contraction. It is caused by the formation of an intestinal cavity in this region in consequence of the pressure and this cavity continually enlarges in the direction of least resistance, *i. e.*, directly dorsally (Figs. 17 and 19) or dorso-posteriorly (Fig. 13) or dorso-anteriorly (Fig. 15) as the case may be. As the size of the pieces from this region increases normal animals are more frequently formed until beyond a certain limit all pieces give rise to normal animals.

At present I desire chiefly to call attention to the fact that the two regions separated by this indifferent region resemble each other in regulation since they usually produce normal animals. We might expect from their position in the body to find the pieces from the post-pharyngeal region predominately "posterior" in function but as a matter of fact they show "posterior" and "anterior" characteristics in equal degree since prepharyngeal and postpharyngeal regions are of the same size. Here again the only satisfactory explanation of the facts is the existence of the early stage of individualization of a new head region somewhere in this postpharyngeal region. The results with small pieces indicate that this condition is not sharply centralized or localized in the region but extends more or less completely throughout its length. Moreover, the remarkable agreement of these different lines of evidence when considered from the point of view adopted in this paper affords an indication of the value of this point of view as a means of interpretation and unification of the mass of data which have accumulated in this field.

I believe that the supporters of theories of regulation based on formative substances or upon "entelechies" will find a certain amount of difficulty in interpreting some of the facts cited here, but discussion of my conception of their bearing is postponed.

As is well known, fission in *Planaria maculata* occurs in a region some distance posterior to the pharynx and the same is

true of the Californian species. My observations have led me to believe, however, that the level varies to a considerable extent. The direct cause of separation seems to me to be a difference in reaction of the two parts; a certain portion of the postpharyngeal region attaches itself firmly while the other parts of the body attempt to move forward. Since the motor power for the forward movement, *i. e.*, the cilia and the margins of the body — is applied not at the anterior end only but throughout the whole length of the part anterior to the attached region the greatest strain will occur just anterior to the attached region and rupture will occur here even in the absence of any preformed zone of weakness. Personally I am inclined to doubt the existence of any such zone of weakness, but I think the facts show very clearly that under ordinary conditions *Planaria* consists physiologically of two zoöids.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
April, 1906.

# THE ORIGIN OF THE ORGAN-FORMING MATERIALS IN THE FROG'S EMBRYO.

T. H. MORGAN.

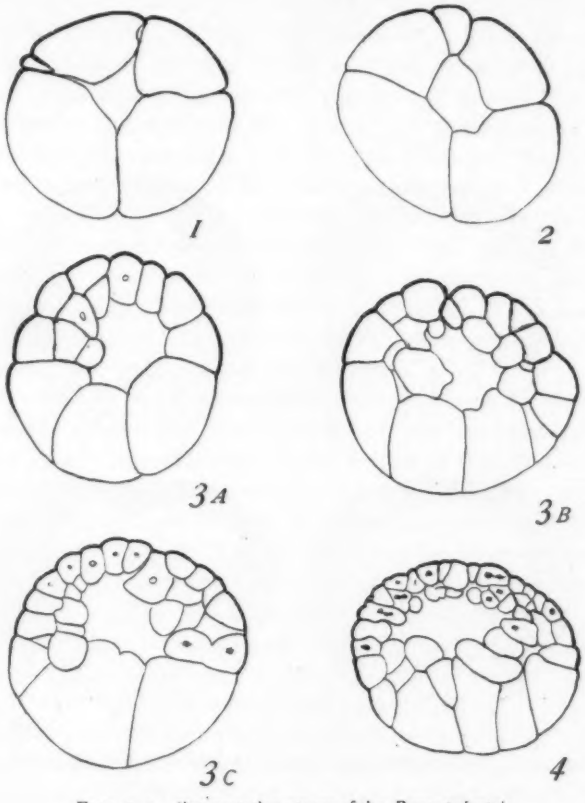
The location of the neural plate of the frog's egg, and of the organs lying immediately below it — the "embryo" in a narrow sense — has been variously determined by different embryologists. Pflüger, Roux, Schultze, O. Hertwig, Morgan, Brachet, H. V. Wilson, Assheton and others have considered this question, but since the literature has been sufficiently reviewed in recent years I shall refrain here from further comment. In the present communication I shall deal with a problem somewhat different from the location of the embryo, namely, the origin of the materials out of which the "embryo" is formed. If I can make good my point it will be seen that the embryo-forming material originates from a part of the egg different from that in which the embryo first appears, hence the location of the embyro on the egg does not give a sufficient answer to the problem of the real source of the materials out of which the embryo develops.

In a series of papers, dealing with the relation between normal and abnormal development of the frog's egg, I was led to certain conclusions in regard to the origin of the organ-forming substances, and in the last paper of the series I dealt directly with this question, basing my conclusions upon a reëxamination of normal development of *Rana palustris*. The present paper deals with the same problem in *Rana sylvatica* and *Bufo lentiginosus*, and includes some new observations on *Rana palustris*.

## Argument.

Briefly stated, my view is as follows: The material, out of which the "embryo" develops, lies in the upper hemisphere, and is transported below the equator of the egg during the segmentation stages. This material forms a ring around the lateral wall of the segmentation cavity, at first above, later below the equator of the egg. The same material can be traced with some prob-

ability to the eight-cell stage, where it exists partly in the upper four cells and partly in the upper ends of the larger lower four cells. In the later segmentation stages, and in the pregastrula stages, this material moves down around the sides of the egg until it comes to lie some distance below the floor of the segmentation cavity. Here it becomes, as described above, the germ-ring, which closing over the lower hemisphere produces the "embryo."

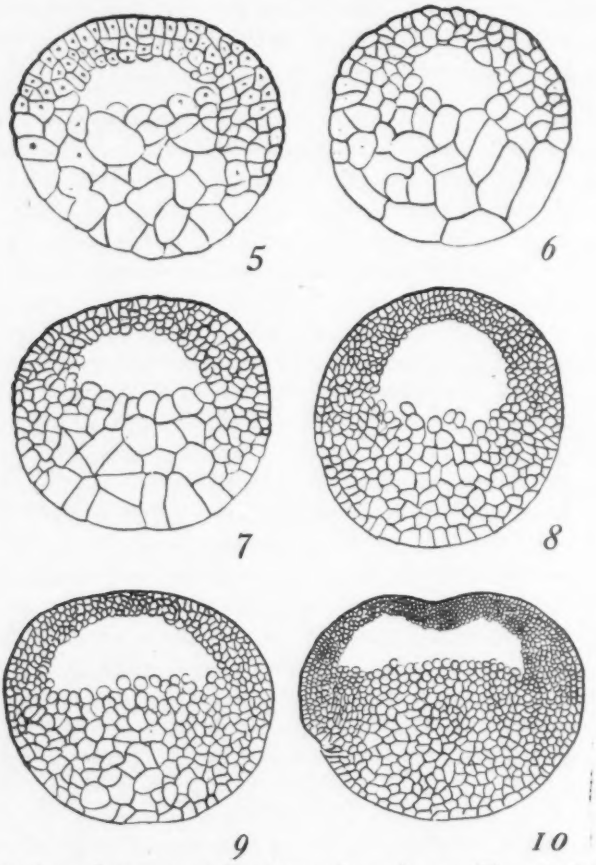



FIGS. 1–4. Segmentation stages of the *Rana palustris*.

The details of these changes, and the evidence on which these statements rest, may now be considered more fully.

## Descriptive.

*Rana palustris.* — Since I now have a more complete series of stages of the eggs of this species, and since I have elsewhere especially considered this form, it may be dealt with first.



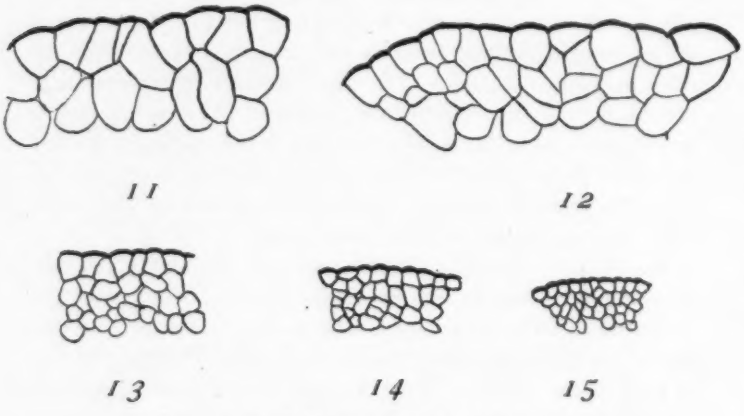

Figs. 5-10. Later segmentation stages and gastrula stage (Fig. 10) of *Rana palustris*.

Fig. 1 is a vertical section of an eight-celled stage. The segmentation cavity is present and is bounded above by the upper four cells (of which two are seen in the section), and at the sides

by the upper ends of the lower four cells. The great thickness of the upper four cells that form the roof of the blastocœl is especially to be noted. The next section, Fig. 2, is a section through a 16-cell stage. The same relations noted for the last stage hold here also. The next three figures are from another set of eggs. Fig. 3, *A*, is a 54-cell stage with six of the cells inside. The cells of the roof have already begun to move out towards the sides, so that the middle part of the roof is much thinner than that of the sides. The next figure, Fig. 3, *B*, is from an egg containing sixty-four cells, of which fifteen are inside. It shows changes similar to those of the last figure. Fig. 3, *C*, is through an egg with 105 cells, of which twenty-six are inside. The shifting of the cells derived from the upper four cells is still more apparent. At this time the top of the roof of the segmentation cavity is much reduced in thickness, while the sides are still quite thick. In the last three figures, the segmentation cavity is very irregular in shape. At first it is deeper in a vertical plane, but in the last stage it is broadening out in a horizontal plane. The last section, Fig. 4, is from an egg belonging to the same set as Figs. 1 and 2. The following six figures, Figs. 5 to 11, belong to still another series.

The fluid in the segmentation cavity is formed by the surrounding cells, and it may be supposed that the thinning out of the roof of the blastocœl is due to loss of fluid, but as the egg as a whole slowly increases in size during this time, the loss of fluid to the blastocœl must be made good by the absorption of water from outside. During the following stages, as seen in Figs. 5–9, the roof becomes very much thinner, as shown strikingly on comparing the sections of the roof at different stages, as seen in Figs. 11–15. It will also be noticed on comparing sections of the earlier and the later segmentation stages, that the side-walls of the segmentation cavity are at all stages very thick compared with the upper wall, and also that the smaller cells of this region are being slowly carried down at the sides of the egg. They furnish the material out of which the "embryo" is later formed. In Fig. 10 the first indications of the dorsal lip are present, as seen to the left. At this stage the smaller cells have pushed down below the equator of the egg on the side at which

the dorsal lip of the blastopore lies. At the same time the lateral wall of the blastocœl has been reduced in thickness, and there can be little doubt that the reduction must be directly due to the movement downwards of its cells. On the opposite side of the section where the ventral lip will form later the down-growth of small cells is less marked, and the lateral wall of the blastocœl is correspondingly thicker. In other words, the ring of embryo-forming material lies at this time obliquely on the egg one side having pushed down further than the other. On the right and left sides of the embryo, as seen when cross-sections are made, the ring shows all intermediate stages between the condition at the dorsal and at the ventral lip.




FIGS. 11–15. Roof of segmentation cavity of *Rana palustris*.

The later history of the gastrulation has been sufficiently described in former papers. In general, the process consists of the steady migration of the embryonic ring over the lower hemisphere. As this takes place the yoke is thrown high up into the segmentation cavity, and the roof of the latter becomes reduced to a thin layer of cells.

The steady decrease in thickness of the roof of the segmentation cavity during the cleavage, pregastrula and gastrulation stages is a constant feature of the development. The decrease in thickness is well shown in the series of figures, Figs. 11–13,

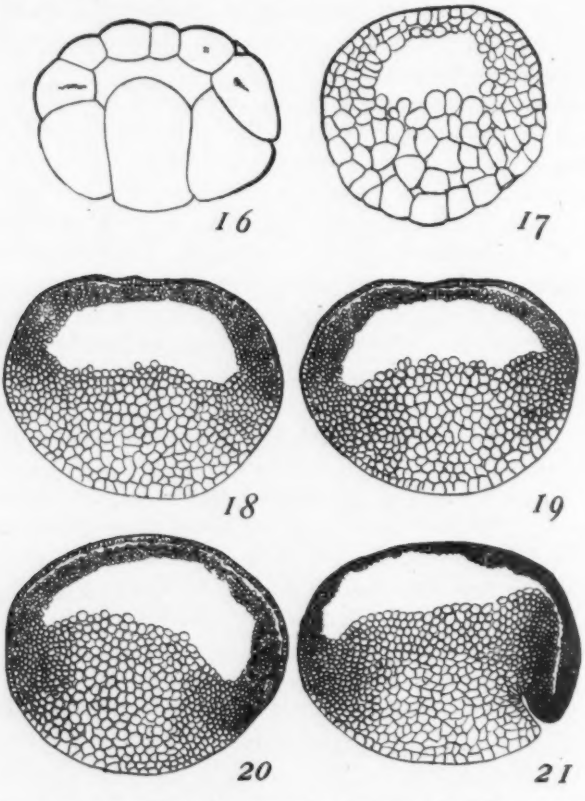
drawn to larger scale than the preceding. Since the cells are dividing during this time without the number of layers in the roof of the blastocœl becoming correspondingly greater — in fact they are fewer in the gastrulation stages — this must mean that as the cells increase in number they push out towards the sides, and increase the material of the embryo-ring, which is, *pari passu*, pushing below the equator of the egg.

*Rana sylvatica.* — Owing to the looseness of the cells of the roof of the segmentation cavity in this species — whether representing a real condition or due to the effects of the preserving reagents I do not know — the preparations show less well the thinning out of the roof of the blastocœl. The first stage figured, Fig. 16, is a section through a 32-cell stage. The inwandering of the upper cells has not yet been accomplished, the segmentation cavity is well developed, and its roof is thick. The upper ends of the four lower cells of the eight-cell stage are, at this time, cut off by the fifth divisions, that usually lies obliquely. In the figure these cells are represented by the two large cells at the sides. They contribute, I think, much of the material that forms the "embryo."

The next figure, Fig. 17, shows a later stage, cut somewhat obliquely. The blastocœl is large, and its roof thinner, but the sides are as thick as before, or nearly so, as shown by superposing the 32-cell stage upon this one. The next two figures, Figs. 18–19, show a broadening of the blastocœl, and thinning of its roof, accompanied by a downgrowth of the small cells around its sides. The next figure, Fig. 20, shows the downgrowth carried further, and the yolk floor correspondingly lifted up. The last figure, Fig. 21, is through the dorsal lip of the blastopore, at the time when the invagination has just appeared. The roof of the segmentation cavity is thinner and continuous at the anterior end with the ectoderm in front of the dorsal lip. This ectoderm is now separated from the cells inside; except in the dorsal lip itself, where ectoderm and mesoderm completely coalesce.[1] A tongue of small cells, running upwards from the blastopore beneath the ectoderm, represents the mesoderm. This mass of cells along with the yolk-cells

[1] Possibly ectoderm cells are added along the middle line to the sheet of mesoderm and give rise to the notochord.

with which it is continuous further inwards, projects upwards at the anterior end of the egg into the blastocœl. On the opposite side of the section, the downgrowth is less developed, and the yolk-floor less elevated.

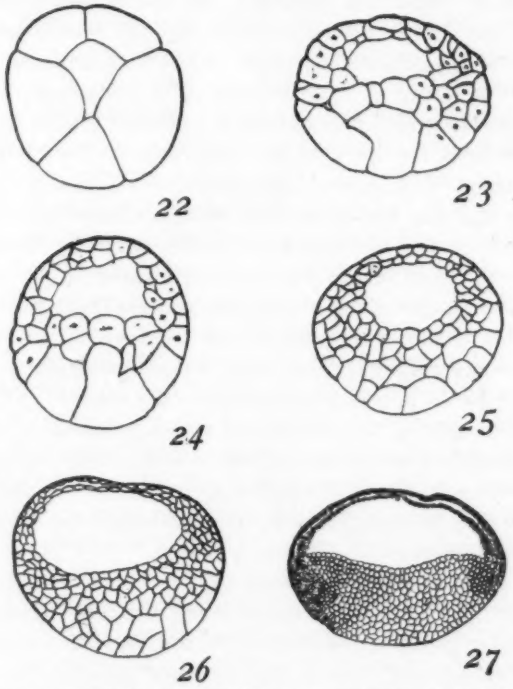



FIGS. 16–21. Segmentation and gastrulation stage (Fig. 21) of *Rana sylvatica*.

The general conditions in this species are the same, in all essential respects, as in the last.

*Bufo lentiginosus*. — The toad, although belonging to another family of the Anura, follows very closely the same method of early development as the frog. In several respects it shows with great clearness some of the early changes that I have described

in the frog's egg. A section through an eight-cell stage is shown in Fig. 22. The thickness of the roof and the sides of the blastocœl, and the extension of the blastocœl far down into the lower hemisphere is very noticeable. In the next stage, Fig. 23, the roof is thinner, and the segmentation cavity extends out more horizontally. The same changes are seen in the next figure, Fig. 24, which is an egg in the same stage of development, but compressed, in cutting, in a plane at right angles to that of the last figure. An older stage is seen in the next figure,




FIGS. 22–27. Segmentation and gastrulation (Fig. 27) stages of *Bufo lentiginosus*.

Fig. 25, where the conditions are much the same. The small embryo-forming cells still lie above the equator. In the next stage, Fig. 26, the segmentation cavity is greatly enlarged, its roof is much thinner, and now the embryo-forming cells lie at

about the equator of the egg. It is evident, when this stage is compared with the last one, that an extensive shifting of the cells must have taken place. This is strikingly shown by superposing the outline of Fig. 22 upon that of Fig. 26. The next stage, Fig. 27, shows important changes to have taken place in the small cells around the sides of the segmentation cavity. These have now pushed below the equator of the egg, and the wall in this region has correspondingly diminished in thickness. This last section is through an embryo that has just reached the gastrulation stage, and although the section passes vertically through the greater diameter of the egg, the blastopore lip lies slightly to one side, and opens in neighboring sections that are not quite so large. The mesoderm cells, containing more pigment than the endoderm cells, form a distinct tongue extending upwards from the level of the blastopore to the floor of the archenteron. The general appearance of the section suggests strongly that the innermost cells have remained stationary, or have even pushed upwards a little, accompanying the general upward movement of the floor of the archenteron.

The later stages of the gastrulation-process in the toad, I have not studied. King has recently given a series of figures showing the later development. They show that the yolk-mass is thrown high up into the blastocoel, especially at the anterior end, so that the anterior part of the archenteron comes to lie higher up than the original floor of the archenteron. It is not improbable that the embryo of the toad lies higher up on the anterior side of the egg than does the embryo of the frog, although the difference is only one of degree.

During the period of closure of the blastopore, the large blastocœl becomes filled by the yolk and the drawing inwards of the yolk brings the dorsal and ventral lips together without, in a sense, their actually growing *over* the lower hemisphere.

## Conclusions.

The large number of cells in the frog's egg at the time when the embryo appears makes it impossible to trace the cell-lineage after five or six divisions, so that we must be content with less refined methods in locating the embryo-forming materials.

Whether the inwandering, or inpulling of some of the cells of the upper hemisphere is only due to the shifting of the cells as they divide, so that the pressure relations are better adjusted, or whether there is a further meaning to be attached to this process cannot be stated. One might be tempted to assign to the cells the function of producing the mesoderm, and the later pushing outwards at the sides of much of the material derived from these cells into a position where the mesoderm appears might be made to give color to this interpretation. However this may be, the experiment of removing the upper four cells shows that some, at least, of the mesoderm comes from other parts of the egg. Whether the same amount forms under these circumstances is too difficult to determine. In the later stages of gastrulation, and at the time also when the neural plate is forming, the mesoderm on the ventral surface increases at the expense of the yolk-cells. These are the yolk-cells that form the tongue of cells that pushes upward into the blastocœl.

In my last paper dealing with the gastrulation of the frog's egg, I discussed the "mechanics" of the downward movement of the material that forms the embryonic ring. Certain points that bear on this question have been noted in studying the three species here described, and may be briefly mentioned. There seems to be a good deal of variation in the extent to which the blastocœl enlarges, not only in different species, but in the same species when eggs from different bunches are compared, and even to a slight extent in eggs from the same bunch. The segmentation cavity appears to be formed as a result of the secretion of material from the surrounding cells, as shown by its occurrence in eggs developing out of water. The egg as a whole increases in size as the segmentation cavity grows larger, indicating that the water absorbed from outside more than compensates for the amount of fluid secreted into the central space.

The thinning of the roof of the segmentation cavity takes place at the time of enlargement of the segmentation cavity and it may appear that this enlargement is the cause of the downward movement of the material; but that this is not the correct interpretation of the mechanics of the process is shown by the following experiment. The top of the segmentation cavity was

opened and partly removed, by means of a needle, at different stages in the cleavage process. Nevertheless, the materials that form the embryonic ring continued to push down at the sides of the egg and a normal embryo was produced. The experiment is open to the obvious criticism that after the removal of the roof, or a part of it, the opening soon closes again; but if the downgrowth were really due to pressure of the blastocœl fluid or even to the pushing of the cells of the roof on each other, the disorganization of the process, that would probably follow, when a part of the roof is removed, must be so great, one would think, that it is unlikely the downgrowth could subsequently take place normally. Still the experiment is inconclusive and does not settle the question.

The two most conspicuous changes that take place during the segmentation and gastrulation stages are the development of the enormous segmentation cavity and its disappearance during gastrulation. We may look upon the *purpose* of the segmentation cavity as a space into which the lower cells may push when the upper cells pass over them. While the presence of the segmentation cavity may facilitate this process, we gain no insight into the origin of the cavity or of its disappearance by a consideration of its purpose. Its formation seems to be due largely, as I have already said, to a secretion of fluid from the surrounding cells; its disappearance is more difficult to explain. The question resolves itself into these alternatives — is the blastocœl fluid absorbed or is it forced out of the egg in the later stages of gastrulation? Since the egg does not decrease in volume or very little, as the yolk surrounds and finally obliterates the segmentation cavity, the fluid would seem to be absorbed. It is true, we might assume that the fluid is squeezed out of the egg and water from the outside absorbed at the same rate, but this assumption only complicates the question, and in the end amounts to the same thing. Admitting that the fluid is absorbed, can this explain the inward migration of the yolk. I think not, because in the first place the process of ingrowth takes place around the sides of the blastocœl cavity and around the lips of the blastopore and not throughout the entire floor of the segmentation cavity, and in the second place there is nothing in the changes that take place

to indicate that they are due only to the absorption of the blastocœl fluid, since the cells that undergo the changes in question do not appreciably grow larger than the others. On the contrary the gastrulation seems to be due to the change in shape and migration of certain cells, and the absorption of the fluid from the blastocœl appears to be no more than the ordinary process of water absorption that takes place throughout the whole period of development.

The evidence that we have at present seems to indicate that the process of gastrulation is due to the activities of the cells themselves, *i. e.*, the "mechanics" of the process can only be explained by an appeal to the response of the cells to certain stimuli. The actual changes that we observe involve a change in shape of certain cells, and our problem resolves itself into determining what stimulus leads to this change in shape, and what physical process is involved in the change. I have pointed out that it seems to me probable that the stimulus is derived from the mutual pressures of the cells, and the change of shape is due to contraction processes in the cells (that lead to their change in shape) that are akin to the same contraction shown by a protozoön, or, in a higher form, by muscle cells. The different behavior of the cells in different parts of the egg must be ascribed to their difference in materials that are derived from the different parts of the egg. After each cell-division, itself apparently a process of local contraction, there must be a rearrangement of the pressure relations in the different parts of the egg.

The formative factors of development can be reduced, from this point of view, to the two generally recognized properties of living matter, irritability and contractility. The stimulus that arouses the irritability is the pressure relation of the cells; the time at which each cell is effected by this stimulus will depend on its material composition, and it responds by its contraction. If this interpretation of the formative changes is correct it refers the process of development to two physiological properties of living matter, and not directly to other known physical properties of organic matter. What the physical bases of irritability and of contractility are remains for the future to decide. They may be

simply complexes of known physical factors, or they may be physical properties of organic matter that are not met with in inorganic compounds. Whatever their ultimate nature it would be a distinct gain if we could prove that most of the phenomena of early development, the so-called formative changes, are due to these two properties of living substance.

# A NOTE ON THE SUSCEPTIBILITY OF SEGMENTING ARBACIA AND ASTERIAS EGGS TO CYANIDES.[1]

A. P. MATHEWS.

The discovery by Lyon[2] that after fertilization sea-urchin eggs show definite periods of resistance and susceptibility to the action of cyanides is of such general interest that it is important to establish the exact period at which eggs are most sensitive and to discover whether other eggs also show similar periods of susceptibility. Lyon found that the eggs were most easily killed about the time of the first cleavage. He was in doubt as to the exact period, but thought it came just after the cleavage, whereas immediately preceding cleavage the egg was very immune. He made no sections and was not entirely certain with just what processes in the egg these periods of immunity and susceptibility coincided. Spaulding[3] tried similar experiments using ether and acids. The ether especially gave a very sharp result and showed great susceptibility immediately *preceding* segmentation. The acid, as might be anticipated from the various actions it possesses, gave a far more complicated series of phenomena. It seemed desirable to ascertain exactly the period of susceptibility to the cyanides and to discover if possible what series of changes within the egg corresponded with the periods of susceptibility and immunity. A study was accordingly made of the living eggs of *Arbacia* and *Asterias* and I have reëxamined a series of sections of *Arbacia* eggs made several years ago, in which the eggs were preserved in sublimate-acetic at definite intervals after fertilization. I also endeavored to repeat Lyon's observations on *Arbacia* using *Asterias Forbesii* (?) eggs. Some very fine material was gathered about the middle of September at Woods Holl.

[1] Laboratory of Biochemistry, University of Chicago, and the Marine Biological Laboratory, Woods Holl.

[2] Lyon, *American Journal of Physiology*, VII., 1902, p. 56; XI., 1903, p. 52.

[3] Spaulding, BIOLOGICAL BULLETIN, VI., 1904, p. 224.

The sections, the living material and the observations on *Asterias* indicate that the period of great susceptibility is *immediately before and during segmentation* and that just after segmentation great resistance prevails.

In the following table I have placed in parallel columns one of Lyon's results, and the phenomena as shown in sections of the eggs. There is one objection to comparing different series of eggs in that the temperature may not have been the same in the different experiments, and the periods will not exactly coincide. However the eggs of *Arbacia* develop very uniformly and nearly always the first cleavage comes in between fifty and sixty-five minutes after fertilization.

TABLE I.

| Minutes After Fertilization. | Susceptibility to Cyanide | Phenomena as Shown in Sections and Living Material |
|---|---|---|
| 0 | Slight. | Egg at rest. |
| 0–10 | Slight increase in susceptibility. | Sperm penetrating egg. Aster very small. Meets egg nucleus in 8–12 minutes. |
| 10–25 | First period of susceptibility. | Fusion of nuclei. Great growth of aster. Caterpillar stage. Rays throughout cell. |
| 30–48 | Progressive increase in immunity. At end most immune. | Pause stage and retrogression of aster. Large rays fade out. Enormous growth of nucleus. |
| 48–60 | Second period of susceptibility. | Nuclear wall fades about 45–50 minutes after fertilization. Tremendous growth of asters follows At 60 minutes chromosomes beginning to separate. |
| 60–65 | Susceptible. | Segmentation completed. |
| 65–70 | Immune. | Retrogression of asters. |

It will be seen from Lyon's experiments that as a rule his period of susceptibility came about fifty-five minutes after fertilization or even five minutes earlier. This period is certainly just before segmentation.

It is clear from this table that the period of great susceptibility coincides with the development of the asters; and the period of greatest immunity coincides with the retrogression of the asters and the development of the nucleus. Thus the first decrease in immunity coincides with the time when the sperm aster is developing. This reaches its maximum about 20–25 minutes after

fertilization. There then ensues a period of retrogression of the aster, the astral radiations fade out, and the nucleus grows enormously. This is the immune period. At the end of that time the nuclear membrane disappears very rapidly and a great growth of the aster takes place, leading up to the first division. This is the second susceptible period. Following segmentation, the nucleus reforms. The asters again decrease in size, and a second period of resistance occurs. This is, however, short, as the second division comes very quickly.

I have attempted to confirm these observations by studying the living *Asterias* egg. I got a very fine lot of *Asterias* eggs, nearly the whole starting maturating within five minutes after shedding. They were fertilized very soon after the germinal vesicle had begun to disappear and transferred at ten-minute intervals (at five-minute intervals during the extrusion of the polar globules and segmentation) to *m*/100, *m*/50 and *m*/25 sodium cyanide solutions. After remaining in the cyanide solution for periods of one, two and three hours they were transferred to fresh seawater, which was repeatedly changed, and were then left to develop.

The results were very unsatisfactory. No sharp periods of susceptibility could be discovered in which the majority of the eggs were killed in the two-cell stage as in *Arbacia*. This failure could not be attributed, I think, to lack of uniformity in the development of the egg, since they segmented with marked uniformity. In practically all cases development proceeded after removal of the cyanide, although there were in some cases marked differences in the appearance of the embryos in lots introduced into the cyanide at different times. Those eggs put in the cyanide immediately after segmentation formed swimming gastrulæ which were fairly normal. Eggs introduced into the cyanide immediately before segmentation had the larger number of dwarf and irregular blastulæ and disintegrating eggs. Frequently the eggs after removal from the cyanide did not segment for several hours and then broke at once into a mass of spheres, some of which died, while others formed the embryo.

The resistance of the eggs was remarkable. One series was left for three hours in *m*/25 cyanide, and the great majority of the

eggs in every lot formed swimming embryos when restored to sea-water.

While the results were thus not sharp and decisive, as in *Arbacia*, and the resistance of the egg to the cyanide very much greater, the experiments were generally harmonious in showing more abnormal embryos in the period just preceding segmentation. In the one exception there were no clear differences between the eggs introduced into the cyanide at different periods.

Why the *Arbacia* eggs are more susceptible when the asters are developing is of course entirely obscure. At this period it is probable, from unpublished observations, that an oxidase escapes from the nucleus and exerts its action upon the cytoplasm. It is possible, also, that at this period the reducing substance in the centriole becomes more active. By an increase in activity is meant an increase in combining power. It may be, therefore, either that the cyanide prevents the oxidase from exerting a necessary action, or it combines with the active centriole and destroys its action, whereas it does not combine with the inactive centriole substance. Further investigation is necessary to decide whether either of these suggestions is the correct explanation. The great resistance of the eggs of *Asterias Forbesii* to the cyanide cannot be explained without further experiment.

# A NOTE ON THE STRUCTURE OF THE LIVING PROTOPLASM OF ECHINODERM EGGS.[1]

A. P. MATHEWS.

From a study of the living protoplasm of several different echinoderm eggs Wilson[2] came to the conclusion that the structure was alveolar, thus confirming Bütschli. During the past summer in connection with the study of the chemical basis of mitosis I had occasion to repeat his observations and have been led to a somewhat different conclusion.

I examined the living eggs of *Arbacia punctulata* and *Asterias Forbesii* under slight compression, using Leitz $\frac{1}{12}$ oil immersion and ocular 4.

In my opinion the phenomena one observes in the living fertilized egg show that the structure of these eggs is not correctly represented by the term alveolar. The protoplasm seemed to me to consist of a perfectly clear, homogeneous matrix in which no trace of structure could be seen, but which contained a vast number of small granules. These granules differ somewhat in size, those near the periphery of the egg being somewhat larger than at the center, and throughout the egg, but particularly near the surface, a great number of very minute granules or microsomes may be seen.[3]

The description of the protoplasm agrees with that given by Wilson except that he regards these granules as alveoli filled with fluid. That they are more correctly regarded as granules in a homogeneous matrix may be shown in a variety of ways.

For example, the granules may be isolated in sea-water if the egg is first treated with sodium sulphate or sodium iodide, $\frac{5}{8}N$, or even in some cases with neutral red. The granules after such

[1] From the Marine Biological Laboratory, Woods Holl.

[2] Wilson, *Journal of Morphology*, XV., 1899, p. 6.

[3] These observations confirm those of F. R. Lillie on the *Chætopterus* egg (Lillie: Personal communication) and of E. P. Lyon on *Arbacia* (see Lyon: Amer. Jour. of Physiol., 1906, vi).

treatment do not swell and disappear as they ordinarily do on crushing the egg, but persist as granules and may be separated from their surrounding matrix. They then acquire a very active Brownian movement and float off as granules.

That they exist as granules in the egg is shown also by their behavior during mitosis and in the centrifuge. In *Arbacia*, the granules are some of them pigmented. During mitosis, these granules do not remain evenly distributed over the surface of the egg, but collect along the furrow of segmentation. Also when the astral centers are formed the granules either move away from the neighborhood of the asters, as has been described for other eggs, or else they are dissolved in these areas. At any rate near the asters and spindles only the clear protoplasm without granules is to be found.

Furthermore, when the large astral radiations extending to the surface of the egg are formed, it may be seen that these radiations consist of the clear homogeneous matrix. The granules are arranged in rows between the broad clear rays. When oxygen is taken away the granules move in again where the rays were.

Further evidence is obtained if the eggs are centrifugalized in the method discovered by Lyon at Woods Holl, this summer. By centrifugalization the granules are separated into zones of granules, as has been described by Lyon and Lillie and the clear protoplasm forms one zone by itself.

The clear protoplasm which forms the matrix in which the granules are, is oftentimes extremely viscid. This is shown in several ways. The granules possess, as long as the egg remains normal, no Brownian movement, showing that they must be in a viscid matrix. It is only when the egg is partially liquefied that they acquire Brownian movement. The viscidity is also shown by observations on living star-fish eggs partially deprived of oxygen. Long viscid strands can then be seen extending between the blastomeres, with here and there granules embedded in them.[1] If these eggs are beaten with a rod, it is frequently possible to draw the protoplasm out in long clear threads.

[1] See Mrs. Andrews : Journal of Morphology, 1896, xii, p. 367.

I do not mean to imply that these granules are hard insoluble granules because many of them, as Wilson has pointed out, appear more fluid than solid, but I mean that the actual structure of protoplasm is more clearly and accurately represented as a clear homogeneous viscid matrix, in which oil globules and all sorts and sizes of granules are embedded than as an alveolar structure.[1] An alveolus is a hole in a matrix. It is not the hole, but the matrix, which in my opinion is the important part of protoplasm.

If one seeks for a substance most nearly resembling protoplasm in structure, raspberry jam, were it colorless, would fairly accurately represent what one sees — a clear sticky matrix in which are a multitude of seeds.

In none of the eggs while living could I see any trace of spindle fibers or of fibers at all like those seen in sections. When the egg is fixed, the proteid colloids which in solution constitute the larger part of the clear viscid matrix, are precipitated in a granular form. In other words, the clear matrix appears to represent a colloidal solution in which our fixing agents throw down a precipitate. The appearance of rays seen in fixed preparations is quite different from appearances in the living egg. In the living egg the clear protoplasm sends off radiating broad strands toward the periphery of the cell. In fixed preparations the rays appear a great deal stiffer and much finer, not so broad as the clear bands although they occupy the position of the living radiations.

These observations indicate very clearly to my mind that the protoplasm of these eggs is essentially a viscid colloidal solution something like gum arabic in viscidity and embedded in it are precipitated or undissolved granules. It may be regarded as a partially precipitated, colloidal solution and the ease with which the granules may be made to dissolve in the clear matrix, indicates, I think, that the amount of precipitate and number of these granules must be constantly changing. Protoplasm appears to be in fact a two-phase colloidal system, undergoing spontaneous chemical change, and surrounded by a semi-permeable membrane.

The changes the granules undergo in the living egg when the latter is compressed or burst are extremely difficult to follow and

[1] From *Asterias* eggs large quantities of an oil may be separated.

interpret. The granules fade out or seem to dissolve in whole or in part in the viscid matrix. This matrix with the dissolved granules, when in contact with water, rounds itself up as if oily and the crushed protoplasm at first as it flows from the egg breaks into a mass of spheres consisting of viscid matter with dissolved granules, and these spheres then ultimately dissolve almost completely in the water.

The speed of solution of the granules is extraordinary. In some cases where the egg was too much compressed by the coverglass and lens, in a few seconds, almost all the granules in the egg disappeared.

These granules all stain in the basic dyes *intra vitam*, and never in acid. I found, however, that if eggs which have died in the ovary are brought into the dyes, all the dead eggs stain in the acid dyes; all the living in the basic. A change in staining reaction, probably coincident with a change in reaction from alkaline to acid, thus occurs at death.

If eggs in which the granules are stained with any basic dye be crushed, it will be observed that the color disappears when the granule dissolves. What becomes of the color is a puzzle. It does not stain the matrix nor appear to color the sea-water. It may be that it is destroyed by the chemical change taking place.

The extreme sensitiveness of the granules, and the abruptness with which the change in appearance of the protoplasm occurs, suggests that possibly this is a physiological process to be correlated with some of the vital phenomena, *i. e.*, contractility.

Dr. R. G. Davis examined, at my suggestion, the chemical composition of the star-fish egg. He succeeded in isolating from the eggs a large amount of an oil, fluid at ordinary temperatures. The chemical nature of this oil has not been determined. I think it possible that many of these granules represent that oil. The oil is very likely partially saponified and contains a small amount of fatty acid. By the dissociation of this acid the drop or oil globule becomes electro-negative. It is in this way the globule acquires the power of staining with basic dyes, since the insoluble soap is formed. The quick disappearance of the granules when compressed, giving one the impression that they are composed of a fluid which mixes with the viscid matrix, and

their quick fusion under the influence of ether, also bear out this interpretation. It is not impossible that ordinarily the granules or oil drops are in combination of a peculiar kind with basic proteids of the protoplasm, the whole forming a highly unstable and complex compound. The chemical composition of these granules is being studied more closely.

# PRELIMINARY REPORT ON THE EMBRYOLOGY OF CRYPTOBRANCHUS ALLEGHENIENSIS.[1]

BERTRAM G. SMITH.

Although the eggs of the hellbender, *Cryptobranchus* (*Menopoma*) *allegheniensis*, have been eagerly searched for by embryologists, a few unfertilized eggs only have hitherto been obtained. Aside from a brief description of an embryo in an advanced stage by McGregor ('96), there is no record of any observations on the development. The manner in which the eggs are fertilized has not been described.

This gap in our knowledge of comparative embryology is serious, particularly as very little is known about the development of the two other members of the family[2] Amphiumidæ: the giant salamander of Japan, *Cryptobranchus japonicus* (*Megalobatrachus maximus* Schlegel), and the American *Amphiuma means*.

During the early part of September, 1905, in northwestern Pennsylvania, I was so fortunate as to obtain fertilized eggs of *Cryptobranchus allegheniensis* in abundance, both from specimens in captivity and from the natural habitat of the animal.

## I. The Sexual Elements.

A. *The Egg.* — The egg proper is perfectly spherical when fresh, about the size of a large pea, and bright yellow in color — a rather deep yellow at the lower pole, grading to a pale yellow at the upper. There is no black pigment such as is found in the eggs of most amphibians. A very thin transparent membrane, the vitelline membrane, quite inconspicuous in fresh material, closely invests the egg. Each egg, with its vitelline membrane, floats in a clear fluid within a hollow sphere or capsule about the size of a large grape, formed by the thick gelatinous outer

[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 105.

[2] Gadow's classification is followed ('01).

envelope, the secretion of the oviduct. This outer egg envelope is produced at opposite poles of the capsule to form a slender cord connecting the eggs in a string (Fig. 1). The envelope is

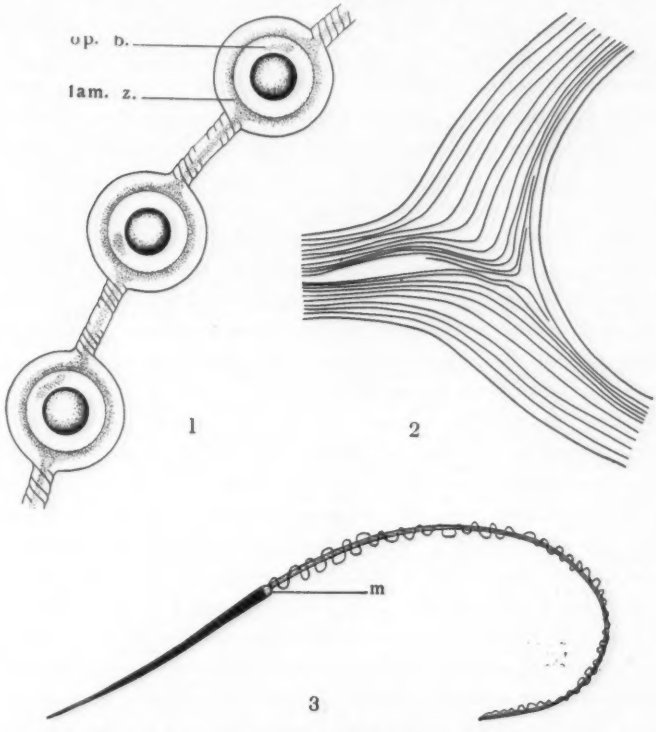



FIG. 1. Eggs and egg envelopes of *Cryptobranchus*, natural size. *op.b.*, opaque body; *lam.z.*, lamellar zone of envelope.

FIG. 2. Optical section (longitudinal) through lamellar zone of envelope in region of junction of egg capsule with connecting cord. Magnified about thirteen diameters.

FIG. 3. Spermatozoön, magnified about five hundred diameters. *m.*, middle piece.

perfectly transparent when fresh, except that wherever viewed tangentially its inner layers have a milky appearance, represented by the shaded zone in Fig. 1, and due to a fine lamellar structure sketched in optical section, with a magnification of about thirteen diameters, in Fig. 2. The misty appearance is

caused by the diffusion of light passing through these concentric layers tangential to their inner surfaces. The core or axis of the connecting cord also has the same milky appearance, due to a continuation of the lamellar structure.

The outer surface of the capsule and cord is, to ordinary observation, perfectly smooth and regularly rounded when the material is in fresh condition. But a careful examination, especially with the aid of a lens, reveals a delicate longitudinal striation due to fine parallel grooves or scratches separated by nearly equal intervals. This structure is too minute to be represented in Fig. 1. In some portions of the string the striæ have a slightly spiral direction about the axis of the cord and capsule. The striæ appear as they would if made by the scratching of the surface of the envelope against the teeth of a fine comb; perhaps they are caused by friction of the envelope against papillæ of some sort in the lower portion of the oviduct or in the cloaca, while the gelatinous material is yet soft.

After the eggs have been in water for several days, or after preservation in formalin or alcohol, the outer layer of the egg envelope becomes cast into conspicuous wavy folds or wrinkles of a different nature from the striæ noted above. These folds appear first at the ends of the connecting cord adjacent to the egg capsule and have here a strongly marked spiral arrangement, suggestive of the chalazæ of the hen's egg (see Fig. 1); later the folding may extend over the capsule and the middle of the cord, but seldom with so regular a spiral arrangement in these regions. Sometimes the spiral is continuous from one capsule to another. As a rule the spiral is constant in the direction in which it extends about the axis in all portions of the cord and capsule.

The inner layer of the lamellar core of the cord in some cases exhibits a marked twisted or spiral arrangement like that of the inner portion of the cord connecting the eggs of *Ichthyophis* as described by the Sarasins ('87–'93).

The following measurements were taken:

| | | |
|---|---|---|
| (1) | Diameter of egg proper, | 7 mm. |
| (2) | " " " with envelopes, | 18 " |
| (3) | " " connecting cord, | 5 " |
| (4) | Distance from one egg to another, measured from center to center, | 3 cm. |

These figures represent the average of several measurements, the range of variation of the first dimension being small, of the others considerable, in eggs from the same parent. The egg proper ordinarily sinks to the bottom of the fluid which surrounds it, so that when viewed from above it is magnified by the lens-shaped capsule so as to appear considerably larger than is indicated by direct measurement.

Occasional strings of egg capsules are found without any eggs enclosed. These empty egg envelopes have the same structure as the others, and usually occur as a portion of a string of capsules the remainder of which contains eggs. A few of the empty egg capsules are double, formed by the union of two capsules without a connecting cord; in such cases the cavities of the two capsules are separated only by a very thin gelatinous septum.

In fertilized eggs in an early stage of development, preserved in formalin without removal from their envelopes, I found floating in the liquid between the egg and its envelope a fairly large irregular and slightly opaque mass, in appearance like a faint white cloud (see Fig. 1). This mass had not been noticed in the capsule of the living egg, probably because it was then transparent. Under the microscope it was found to consist of a clear gelatinous matrix in which were embedded numerous large round cells each with a very large nucleus and often with what appeared to be a large vacuole. By the use of a dissecting lens the individual cells could be seen while still within the envelope. In some egg capsules they occur diffused throughout the liquid of the cavity, not aggregated in a mass to form an opaque body. Numerous spermatozoa are present in the opaque body, but they also occur floating in the surrounding liquid and embedded in the egg capsule. Whether the opaque body is present in unfertilized eggs I have not at present the material to determine. The unfertilized eggs of *Cryptobranchus allegheniensis* have been briefly described by Reese ('04) but without mention of any such feature as the opaque body described above. Both opaque body and spermatozoa regularly occur in egg capsules that do not contain eggs. The origin and function of the cells of the opaque body are at present problematical.

The eggs of *Cryptobranchus allegheniensis* bear a rather close resemblance to those of *C. japonicus* as described by Sasaki ('87), Kerbert ('04), Ishikawa ('04) and de Bussy ('05). Other amphibians whose eggs are fastened together like a string of beads are *Amphiuma* (Hay, '88 and '90), *Desmognathus*, *Ichthyophis*, *Hypogeophis* (Brauer, '97), and *Alytes*. The eggs of *Bufo* are arranged in strings but without the marked constriction of the envelope between the eggs characteristic of the other forms mentioned.

B. *The Sperm.* — The spermatozoön (Fig. 3) is of considerable size, about 225 $\mu$ long, but with an unusually small middle-piece. The head of the spermatozoön, excepting the tip or acrosome, stains deeply with Conklin's modification of Delafield's hæmotoxylin. The head is very long and slender, tapering gradually to the extremely fine-pointed acrosome, enabling the spermatozoön to pierce easily through the vitelline membrane. The tail-piece is provided with an undulating membrane, bordered with a convoluted filament.

The spermatozoön of *Cryptobranchus allegheniensis* resembles that of *C. japonicus* as figured by Ishikawa ('04). It is also quite similar to the spermatozoön of *Amphiuma* (McGregor, '99), but the latter has a larger middle-piece and a slightly barbed acrosome.

## II. Breeding Habits.

A. *Method of Fertilization.* — A newly-captured female was isolated in an aquarium not previously occupied by any other specimen. A large number of eggs were laid, which contained spermatozoa within the egg capsule; moreover the subsequent development of these eggs proved them to have been fertilized. Hence there can be no doubt that in *Cryptobranchus alleghenicnsis*, as in all other Urodeles so far as known, fertilization, unlike that of the *Anura*, is internal. The mode of transference of the male element to the cloaca of the female has not been ascertained.

Nothing conclusive has been established concerning the method of fertilization of the nearest relative of *Cryptobranchus alleghenicnsis*, the giant salamander of Japan. In the case of *Amphiuma*, Davison ('94 and '95) believed a transference of spermatozoa

from male to female to take place by means of an apposition of the lips of the two cloacæ, basing this belief on what appears to me insufficient evidence; such a process is moreover not in harmony with known methods of fertilization among the Urodeles, in which fertilization is effected by means of spermatophores and without direct cloacal contact (Jordan, '91 and '93). Internal fertilization is said to occur in the Gymnophiona, *Ichthyophis* and *Hypogeophis*, as well as in the Urodeles.

B. *Breeding Season.* — Fertilized eggs were laid by a specimen in captivity on September 6, and another spawning by the same female took place during the night of September 7–8. Eggs in the first cleavage stage were found in the natural habitat of the animal on September 7, and another spawning of eggs in an advanced stage of segmentation was found in the same habitat on September 8.

Townsend ('82) records the laying of some eggs by a specimen in captivity during the month of August. McGregor ('99) states that the eggs are deposited in August and September.

No direct observations as to the time when the females are fertilized were obtained; but male specimens killed during the early part of September were found to have the vasa deferentia distended by a large quantity of seminal fluid containing an abundance of ripe spermatozoa. According to McGregor ('99) "the sexual union must occur very near or at the time of egg-laying, for the female is devoid of spermathecæ, and the spermatozoa do not ripen until late in August."

Eggs of *C. japonicus* were found by Sasaki ('87) in August. Kerbert ('04) records the spawning of a specimen in captivity during the night of September 18, 1902, and again on September 19, 1903. DeBussy ('05) reports that eggs were laid by Kerbert's specimen during September 14, 1904, and several days following. According to Ishikawa ('04) the eggs are laid principally during the latter half of August, but also in September. Hence the breeding season coincides with that of *C. allegheniensis*.

Eggs of *Amphiuma* in an advanced stage of development were found by Hay ('88 and '90) on September 1. Davison ('94 and '95) states that the eggs are deposited in August or September, but without giving the data upon which this statement is based.

He found a viscid substance containing spermatozoa exuding from the vent of a male specimen in May (April, according to his earlier paper), and concluded that this month is the natural season for fertilization.

The occurrence of the breeding season of *Cryptobranchus* in the fall is in marked contrast to the habits of nearly all other Urodeles, since they lay their eggs in the spring. While the significance of this unusual breeding season is not readily apparent, it is, in the case of *C. allegheniensis*, in at least one respect adaptive. The animal is an inhabitant of streams that during spring and early summer are subject to frequent and destructive freshets, which would probably be disastrous to the development of eggs like those of *Cryptobranchus*. During late summer and fall the streams are shallow and the water comparatively quiet; floods are of rare occurrence. These factors do not affect the other amphibians of the region in the same way, since they are inhabitants of ponds not seriously disturbed by floods, and on account of the more abundant rainfall better adapted for breeding-grounds in the spring than later in the year. There is every reason to believe that these climatic conditions have been of long duration. Probably the same conditions prevail with regard to *C. japonicus*, which is an inhabitant of mountain streams similar to those in which *C. allegheniensis* occurs. In this connection Professor Jacob Reighard informs me that the increase of spring freshets in Michigan during recent years, aided no doubt by other effects of lumbering operations, has nearly caused the extinction of the grayling, a fish that breeds in the spring, and was formerly abundant; the trout, which breeds in the fall, now thrives in the same streams. These facts indicate the selective value of the factors mentioned, and support the view taken with regard to *Cryptobranchus*.

C. *Breeding Habitat.* — Eggs of *Cryptobranchus* were found in shallow water in what had once been the main channel of a large stream, but through which now only a portion of the water, separated from the main channel by an island, flows. This old river channel is extremely rocky, with a considerable incline, so that the shallow water alternately forms pools and rapids. Judging from the number of specimens seen, the locality is a

favorite haunt of *Cryptobranchus*. Adult hellbenders were seen in other portions of the stream in deeper water, but in no other situations were eggs found.

The locality described above bears a strong resemblance to the habitat of *C. japonicus* as illustrated by Ishikawa ('04).

D. *Habits of Oviposition.* — Two separate spawnings of eggs were found in the natural habitat of the animal, besides eggs laid on two different occasions by a specimen in captivity. On account of differences in the method of disposal of the eggs, these spawnings are best described separately.

The eggs found on September 7 were lying in gently flowing water about 2–4 inches deep, on a gravelly and stony bottom, within a space about 6 feet in diameter nearly enclosed by some large rocks which projected a foot or more out of the water. The eggs were arranged in long festoon-like strings, scattered over an area of about 2 x 5 feet. In a few places the eggs were grouped in masses, but these masses might readily be resolved into strings. All the eggs present were included in a very few strings; one string contained 27 eggs. Evidently the eggs had not been disturbed since being laid. One hundred and ten eggs were counted in full view; but some other eggs had sunk down into crevices between and beneath stones; these brought the entire number up to 135. The eggs lay for the most part in direct sunshine, and the shallow running water furnished them with abundant aëration. No adult hellbenders were seen in the immediate vicinity.

The eggs were conspicuous because of their size and number, and were really beautiful objects, on account of the regularity of their form, the festoon-like manner of their arrangement on the pebbly bottom, the bright yellow of the yolk, and the perfect transparency of their gelatinous envelopes except where bordered with a delicate misty gray.

On September 8 another spawning of eggs, perhaps 50 in number, was found not far from the spot where eggs were discovered on September 7. The eggs were in water 3–5 inches deep, on the down-stream side of a rock, and most of them in a shallow cavity about 16 inches wide and extending back about 8 inches under the rock. From the appearance of the surround-

ing and underlying gravel this hollow looked as if it had been dug out by some animal. The eggs had been disturbed and scattered considerably; the strings were short, as if they had been much broken up, and many separated eggs were found. The eggs were thickly covered with silt. Both the envelopes and the contained eggs were, as a rule, slightly larger than those previously found. No adults were seen in the vicinity.

The eggs obtained on September 6 from a specimen in captivity were found rolled and tangled together in such an intricate manner that they seemed to occur in clusters or masses rather than in strings. The number was estimated at about 80, nearly all in one oblong mass. The solitary female did not appear to care for the eggs in any way.

On September 8 about 300 more eggs were laid by the same female specimen in captivity. The strings of eggs were aggregated in one large mass, but they were not so much tangled as in the case of those laid previously. There were present at the time in the same aquarium three male specimens, but so far as was observed, none of the adults paid any attention to the eggs. When all four specimens were killed a few days later for the purpose of determining the sex by dissection, the stomachs of the males were found distended with undigested eggs.

Upon examination, the female, which during captivity had laid nearly 400 eggs, was found to contain, at a rough estimate, seven or eight hundred more, in a state of development which indicated that had she been allowed to live they would all have been laid during the same season. Evidently the eggs are, in some cases at least, matured and laid in batches of a few hundred at a time.

According to Gadow ('01), *Amblystoma* alone among the Urodeles lays as many as 1,000 eggs in a single season. The facts stated above make it probable that *Cryptobranchus* rivals *Amblystoma* in the number of eggs laid. Kerbert's specimen of *C. japonicus* is reported to have laid 500 eggs in the fall of 1902, and about 900 in 1904. The eggs of *Amphiuma* found by Hay numbered about 150.

The absence of any evidence of brooding habits of either the male or the female *Cryptobranchus* is rather unexpected in view of the possession of brooding habits by closely related forms.

Concerning the nesting and brooding habits of *C. japonicus* Ishikawa says: "Das tier legt seine Eier in tiefe horizontal verlaufende Löcher, in denen das Wasser sehr ruhig ist. Manchmal ist solch ein Loch 10 oder mehr Fuss tief und kaum für das Licht zugänglich. Die Brutstellen für die Eier sind aber nicht immer so tief. Oft fand ich Eier in einem Loch nicht tiefer als 3 oder 4 Fuss. Oeffnet man ein solches Loch, so findet man eine abgerundete Stelle, deren Boden ganz rein gehalten ist. . . . Fast in jedem Loch, wo man von Ende August bis zu Anfang October ein weibliches Tier gefunden hat, findet man einen Eiklumpen. Dieser Umstand lässt schon vermuthen, dass das Tier eine Brutpflege hat wie *Ichthyophis* oder wie so viele andere Amphibien." Kerbert, however, asserts that it is the male that guards the eggs, and states that the sex of his specimens was carefully determined. The only external distinction between the sexes is that during the breeding season the lips of the cloaca of the male are greatly swollen. Ishikawa gives an illustration of an adult specimen of *C. japonicus* lying in a coil about a mass of eggs; Kerbert states of his specimen that the male, after driving away the female and also the small fishes present in the aquarium, crept between the folds of the mass of eggs, or sometimes simply lay down beside them, but in either case he kept the entire mass in motion by a pendulum-like movement of the entire body.

The eggs of *Amphiuma* found by Hay ('88 and '90) in an Arkansas swamp were in a comparatively dry situation, in a small excavation under a log several rods from the nearest water. The female was found coiled about the mass of eggs, protecting them and keeping them moist.

Other Amphibia for which brooding habits have been established are the Urodele, *Desmognathus;* the Gymnophiona, *Ichthyophis* and *Hypogeophis; Alytes* and several other Anura (Wiedersheim, '00). In the cases of *Desmognathus*, *Ichthyophis* and *Hypogeophis*, the female is said to care for the eggs; in the case of *Alytes*, the male.

E. *Adaptation of the Egg to its Environment.*— The egg proper is so soft and fragile that it can sustain only the most careful manipulation without injury; on account of its lack of firmness it soon becomes oblate from the effects of gravity. In view of

the fact that the eggs are laid in running water, the protection of such an egg from shocks and jars due to impacts against the rocks is an exceedingly important matter, and we find it accomplished by an admirable adaptation of the egg envelope. The tough but elastic egg capsule is inflated by osmosis with a liquid in which the egg freely floats. The turgid condition of the envelope increases its efficiency in protecting the egg. While ordinarily the egg sinks to the bottom of the surrounding liquid, and rests lightly upon the membrane beneath, there is so little friction between the egg and its envelopes that the latter may be rotated without turning the egg. A similar adaptation exists in the eggs of other amphibians, and in the eggs of teleosts (Reighard, '93); but it is particularly well shown in the egg of *Cryptobranchus*. The connecting cord is strong enough to support the weight of half a dozen eggs suspended out of water; it stretches greatly during the operation, but contracts to its usual length when the eggs are returned to the water. This elasticity of the connecting cord serves to deaden any shock to the eggs due to the tugging of the current or to the chain catching on to rocks while floating down stream.

The egg envelopes further serve to protect the eggs from little fishes that would otherwise devour them.

If eggs are left for several days undisturbed in the still water of a basin, some of them adhere to the envelopes beneath, interfering with the development. Probably under natural conditions the gentle agitation of the current tends to prevent this, performing for these eggs the same service afforded to the eggs of the hen by the mother when she turns them.

## III. Segmentation.

The perfect transparency of the gelatinous envelopes of these eggs makes them very favorable material for watching the development of the living egg, since the process may be observed without removing the egg from the capsule. The large size of the eggs and the distinctness of the cleavage lines on their upper hemispheres also make them convenient objects for study. By using a mirror both poles of the egg may be watched at the same time, but observations on the lower hemisphere by this method

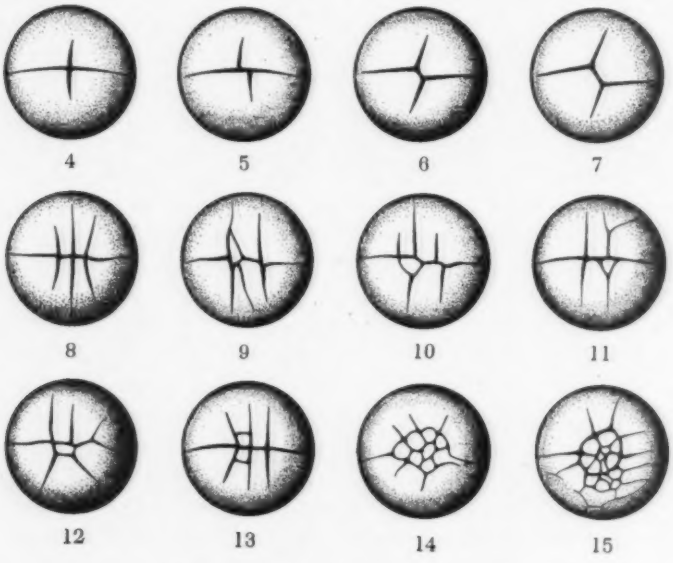
are rather difficult, on account of the faintness of the cleavage lines in this region. Material was preserved at intervals and camera drawings afterwards made of the principal stages; but for the earliest stages the freehand sketches made from living material are sufficiently accurate, and have been used in preference to camera drawings from preserved material.

Some of the eggs found on September 7 were left to develop *in situ* as a check upon those studied under artificial conditions; but so far as cleavage is concerned no difference could be detected between these eggs and those kept in a dish of shallow water.

The abundance of the material at hand made it possible to secure a series of drawings of the segmentation stages which should be fairly representative. The drawings include some of the most regular and geometrically perfect figures that could be found, and probably these may be regarded as typical; but in *Cryptobranchus* as in other amphibians the amount of variation is great. Since departures from the type are not necessarily the result of pathological conditions, they should not be dismissed as abnormalities; for they may be the expression of opposing factors in the development of the egg. Such factors are the proportion of yolk to protoplasm, the qualitative composition of the yolk determining the extent to which it responds to the sorting influence of gravity, and hereditary factors in the protoplasm; variations in these factors affect the manner of cleavage, and according as one tendency or another prevails we may find the typical form or variations from it in definite directions. Hence the figures include representations of eggs in the same stage showing differences in the method of cleavage. There are of course individual differences in the rate as well as in the manner of cleavage.

A. *The First Cleavage Furrow.* — In eggs laid September 6 at about 6:30 P. M., there was so much diversity in the time of appearance of the first cleavage furrow that it is difficult to assign limits. Probably more cases of first cleavage were noticed at about 10 A. M. of the next day — 15 hours after the eggs were laid — than at any other time. In eggs laid during the night of September 7–8 the first cleavage furrows appeared in several eggs at about 5 P. M. of the next day, and cases were numerous at 6:30 P. M. The eggs found on September 7 at 10 A. M. were

in the first cleavage stage; observations on the rate of deposition of silt on their envelopes indicated that they had been laid during the preceding night or late in the afternoon of the day before.




FIGS. 4–15. Early segmentation stages of eggs of *Cryptobranchus*. Freehand sketches from living material magnified about $\frac{1}{4}$ diameters.

FIG. 4. Egg found September 7 at 10 A. M., sketched at 1 P. M. The first cleavage line extends over exactly half a circumference.

FIGS. 5–7 show variations in the second cleavage. Fig. 5, egg laid September 6 at about 6:30 P. M., sketched September 7 at 3 P. M. Figs. 6 and 7, eggs found September 7 at 10 A. M., sketched at 1:30 P. M.

FIG. 8. Egg laid during night of September 7–8, sketched September 9 at 5:30 A. M.

FIGS. 9–11. Variations in third cleavage. Eggs laid during night of September 7–8, sketched September 9; Fig. 9 at 3:50 A. M., Fig. 10 at 2:30 A. M., Fig. 11 at 5:30 A. M.

FIG. 12. Egg laid during night of September 7–8, sketched September 9 at 5:45 A. M.

FIGS. 13 and 14. Fourth cleavage stages. Fig. 13, a later stage of egg represented in Fig. 8, sketched September 9 at 8 A. M.; Fig. 14, a later stage of egg represented in Fig. 9, sketched September 9 at 8 A. M.

FIG. 15. Egg found September 7 at 10 A. M., sketched September 8 at 8:45 A. M. Four nearly equidistant cleavage lines reach almost to the center of the lower pole.

It is not known whether the eggs of a given spawning are all fertilized at the same time, hence some of the diversity in the time of appearance of the first cleavage furrow may be due to a difference in the time of fertilization.

The first cleavage furrow begins as a pit, which gradually elongates, rapidly at first then more slowly. The point of origin of the first cleavage furrow remains always uppermost, even though the envelope is rotated.

B. *The Second Cleavage Furrow.* — This furrow makes its appearance about six hours after the first, which by this time has extended over about half the distance to the equator of the egg. The second cleavage furrow usually cuts the first at right angles, but some variations from this procedure are shown in the figures. (See Figs. 4–7.)

Some time before the appearance of the second groove, the first one appears constricted and narrow in the middle portion, while still broad at the ends. The earliest indication of the second furrow is usually a roughness in the region where the second groove is to intersect the first. The appearance of "Faltenkränzen" — a quivering of the surface with the formation of fine radiating or parallel wrinkles, which extend outward from the cleavage furrow for a moment, tremble, and disappear — a common phenomenon in the cleavage of the amphibian egg, is quite marked at the time of the beginning of the second cleavage furrow. For some time after its appearance the second furrow is much broader, though of course shallower, than the first.

C. *The Third Cleavage Furrow* (see Figs. 8–12). — These furrows mark the establishment of the 8-cell stage about four or five hours after the appearance of the second cleavage groove. Hence the third division is more rapid than the second. At the time when the third furrows are initiated the first furrow has usually reached or passed the equator; the second one is confined to the upper hemisphere.

The third cleavage furrows ordinarily begin as two pits in the first furrow, not far from its point of intersection with the second — the center of the animal pole of the egg — and equidistant from that point. From these pits the third furrows proceed in an approximately vertical direction. The third cleavage furrow seldom

originates from the center of the first furrow, and seldom reaches the lower pole, but extending obliquely in the lower hemisphere joins the second furrow at some distance from the pole (see plate, Fig. 16*B*, line *b*; Fig. 17*B*, lines *b*, *g* and *e*; Fig. 18*B*, lines *b*, *i* and *e*, etc.). Hence the third cleavage furrow is *intermediate between a true meridional cleavage and a latitudinal one*, approaching more nearly the meridional type. This will be made clearer by supposing the point of origin of the third cleavage furrow to be shifted along the first further from the animal pole; and its point of junction with the second to be shifted further from the lower pole; the third groove will then become latitudinal. On the other hand, if the two ends were shifted in the reverse directions, the cleavage lines would become truly meridional. Variations from the customary mode of cleavage tend to confirm the view that it is an intermediate or transitional form; while some third cleavage furrows originate at the center of the animal pole, giving a true meridional cleavage, others come in latitudinally. (See Figs. 9–12.)

D. *The Fourth Cleavage Furrow* (Figs. 13–15). — This comes in about 3 hours later — a briefer interval than that preceding the appearance of the third cleavage furrow. In its typical condition it is parallel to the equator, but close to the animal pole, so that the division is very unequal. Irregularities in this cleavage are very numerous.

E. *Later Segmentation Stages* (see plate, Figs. 16–24). — The later divisions occur with increasing rapidity, but with such irregularity that no definite arrangement of cleavage furrows can be made out. Cleavage proceeds rapidly near the animal pole, much more slowly in the equatorial region, and is greatly retarded in the lower hemisphere. At some time during the second day after the egg is laid the first cleavage furrow reaches the lower pole. At this time the upper hemisphere is cut up into a considerable number of cells. The macromeres continue to be very much larger than the micromeres. While so far as can be determined from a surface study of the egg, the cleavage is undoubtedly holoblastic, a strong tendency toward the meroblastic condition is evident.

Two days after the egg is laid, cell division in the upper hemisphere has advanced until the individual cells can no longer be seen with the naked eye. The macromeres are in general still quite large, but in several instances there was noted a greater multiplication of cells about the lower pole than in the equatorial region (see Fig. 24*B*).

F. *Comparison with Other Forms.* — De Bussy ('05) has described the principal segmentation stages of the eggs of *C. japonicus*. His material lacked eggs in the 2-cell and 4-cell condition; but beginning with the 8-cell stage the later cleavage stages are described and illustrated. A comparison with my own sketches of the segmentation of the eggs of *Cryptobranchus allegheniensis*, made before I knew of the existence of de Bussy's paper on *C. japonicus*, shows, as might be expected from the close relationship of the two forms, a marked similarity in the mode of cleavage. Since de Bussy's paper includes a comparison of the eggs of *C. japonicus* with those of other forms, a similar discussion for *C. allegheniensis* at the present time seems unnecessary. It should be added, however, that the segmentation of the eggs of *Desmognathus*, described by Wilder ('04) and Hilton ('04) also bears a considerable resemblance to that of *Cryptobranchus*.

I take pleasure in acknowledging my indebtedness for encouragement and advice to Prof. Jacob Reighard, at whose suggestion I first looked for the eggs of *Cryptobranchus*, and to Dr. O. C. Glaser, under whose direction the work was carried on after I returned to the university.

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## Explanation of Plate.

All the figures are camera drawings from preserved material. Figs. 16–19 are magnified about three diameters, the remaining figures about four diameters. *A* and *B* indicate upper and lower hemispheres respectively. The small letters serve to identify lines which cross the equator.

Figs. 16–18. Eggs laid during night of September 7–8, preserved September 9 at 2 P. M.

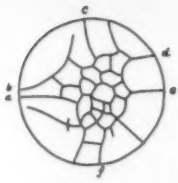
Figs. 19 and 20. Eggs found September 7 at 10 A. M., preserved September 8 at 9 A. M.

Fig. 21. Egg laid September 6 at 6:30 P. M., preserved September 8 at 8:30 A. M. Lateral view.

Fig. 22. Egg laid during night of September 7–8, preserved September 11 at 10 A. M.

Fig. 23. Egg found September 7 at 10 A. M., preserved September 10 at 8 A. M.

Fig. 24. Egg laid September 6 at 6:30 P. M., preserved September 8 at 8:30 A. M.



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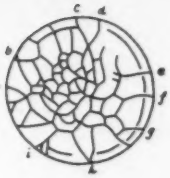
17A

18A



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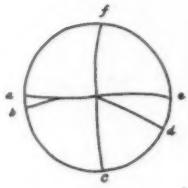

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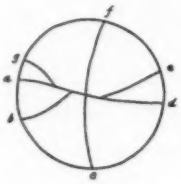

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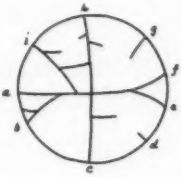

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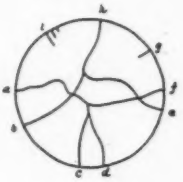

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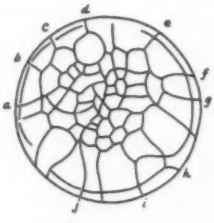

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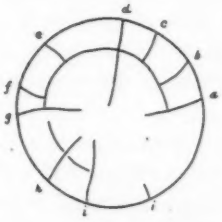

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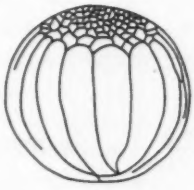

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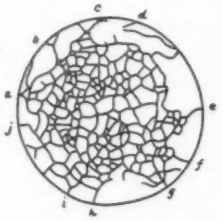

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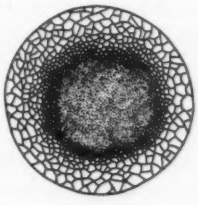

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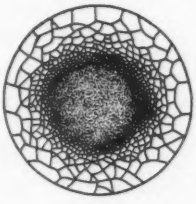

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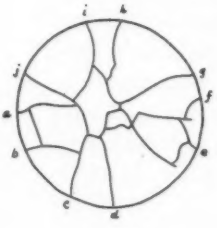

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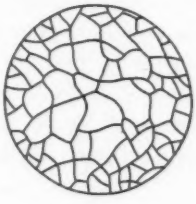

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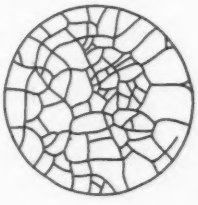

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